4-305883/ P1

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The Patent Office
Cardiff Road
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27 JUL 1998

1. Your reference — 4-30583/P1

2. Patent application number *(The Patent Office will fill in this part)* — **9816281.1**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

NOVARTIS AG
Schwarzwaldallee 215
4058 Basel

Patents ADP number *(if you know it)* 7125457002

If the applicant is a corporate body, give the country/state of its incorporation — Switzerland

4. Title of the invention — Organic Compounds

5. Name of your agent *(if you have one)*

B. A. Yorke & Co.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Coomb House
7 St. John's Road
Isleworth, Middlesex TW7 6NH

Patents ADP number *(if you know it)* 1800001 ✓

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from a earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer "Yes" if:*

a) any applicant named in part 3 is not an inventor , or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

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9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	7
Claim(s)	1
Abstract	
Drawing (s)	

10. If you are also filing any of the following, state how many against each items.

Priority documents	-
Translations of priority documents	-
Statemtent of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
Request for preliminary examination and search *(Patents Form 9/77)*	yes
Request for substantive examination *(Patents Form 10/77)*	-
Any other documents *(please specify)*	-

11. I/We request the grant of a patent on the basis of this application.

Signature B. A. Yorke & Co. Date: 27th July 1998

12. Name and daytime telephone number of person to contact in the United Kingdom: Mrs E Cheetham 0181 560 5847

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Organic Compounds

The invention is directed to the use of a CD25 binding molecule to ameliorate disease symptoms of rheumatoid arthritis.

More specifically the present invention provides in a first aspect the use of a CD25 binding molecule which comprises at least one antigen binding site comprising at least one domain which comprises in sequence, the hypervariable regions CDR1, CDR2 and CDR3; said CDR1 having the amino acid sequence Arg-Tyr-Trp-Met-His, said CDR2 having the amino acid sequence Ala-Ile-Tyr-Pro-Gly-Asn-Ser-Asp-Thr-Ser-Tyr-Asn-Gln-Lys-Phe-Glu-Gly, and said CDR3 having the amino acid sequence Asp-Tyr-Gly-Tyr-Tyr-Phe-Asp-Phe; or direct equivalents thereof to ameliorate disease symptoms of rheumatoid arthritis such as number of tender and swollen joints, degree of tenderness and swelling, or pain.

The use of a CD25 binding molecule according to the invention results in a better disease outcome than the as yet available therapies.

In a second aspect of the invention, a CD25 binding molecule comprising at least one antigen binding site comprising:
a) a first domain comprising in sequence the hypervariable regions CDR1, CDR2 and CDR3; said CDR1 having the amino acid sequence Arg-Tyr-Trp-Met-His, said CDR2 having the amino acid sequence Ala-Ile-Tyr-Pro-Gly-Asn-Ser-Asp-Thr-Ser-Tyr-Asn-Gln-Lys-Phe-Glu-Gly, and said CDR3 having the amino acid sequence Asp-Tyr-Gly-Tyr-Tyr-Phe-Asp-Phe and,
b) a second domain comprising in sequence the hypervariable regions CDR1', CDR2' and CDR3', said CDR1' having the amino acid sequence Ser-Ala-Ser-Ser-Ser-Ile-Ser-Tyr-Met-Gln, said CDR2' having the amino acid sequence Asp-Thr-Ser-Lys-Leu-Ala-Ser, and said CDR3' having the amino acid sequence His-Gln-Arg-Ser-Ser-Tyr-Thr;
or direct equivalents thereof, is used.

Unless otherwise indicated, any polypeptide chain is herein described as having an amino acid sequence starting at the N-terminal extremity and ending at the C-terminal extremity.

When the antigen binding site comprises both the first and second domains, these may be located on the same polypeptide molecule or, preferably, each domain may be on a different chain, the first domain being part of an immunoglobulin heavy chain or fragment thereof and the second domain being part of an immunoglobulin light chain or fragment thereof.

Accordingly, the invention also provides the use of a CD25 binding molecule which comprises at least one antigen binding site comprising either a first domain having an amino acid sequence identical or substantially identical to that shown in Seq. Id. No. 1 in EP 449769 starting with amino acid at position 1 and ending with amino acid at position 117 or a first domain as described above and a second domain having an amino acid sequence identical or substantially identical to that shown in Seq. Id. No. 2 in EP 449769, starting with amino acid at position 1 and ending with amino acid at position 104 to ameliorate disease symptoms of rheumatoid arthritis.

Monoclonal antibodies raised against a protein naturally found in all humans must necessarily be developed in a non-human system e.g. in mice. As a direct consequence of this, a xenogenic antibody as produced by a hybridoma, when administered to humans, elicits an undesirable immune response which is predominantly mediated by the constant part of the xenogenic immunoglobulin. This clearly limits the use of such antibodies as they cannot be administered over a prolonged period of time. Therefore it is particularly preferred to use single chain, single domain, chimeric or humanized antibodies which are not likely to elicit a substantial allogenic response when administered to humans.

In view of the foregoing, a more preferred CD25 binding molecule for use to ameliorate disease symptoms of rheumatoid arthritis is selected from a chimeric anti-CD25 antibody which comprises at least

a) one immunoglobulin heavy chain or fragment thereof which comprises (i) a variable domain comprising in sequence the hypervariable regions CDR1, CDR2 and CDR3 and (ii) the constant part or fragment thereof of a human heavy chain; said CDR1 having the amino acid sequence Arg-Tyr-Trp-Met-His, said CDR2 having the amino acid sequence Ala-Ile-Tyr-Pro-Gly-Asn-Ser-Asp-Thr-Ser-Tyr-Asn-Gln-Lys-Phe-Glu-Gly, and said CDR3 having the amino acid sequence Asp-Tyr-Gly-Tyr-Tyr-Phe-Asp-Phe and

b) one immunoglobulin light chain or fragment thereof which comprises (i) a variable domain comprising in sequence the hypervariable regions CDR1', CDR2' and CDR3' and (ii) the

constant part or fragment thereof of a human light chain; said CDR1' having the amino acid sequence Ser-Ala-Ser-Ser-Ser-Ile-Ser-Tyr-Met-Gln, said CDR2' having the amino acid sequence Asp-Thr-Ser-Lys-Leu-Ala-Ser, and said CDR3' having the amino acid sequence His-Gln-Arg-Ser-Ser-Tyr-Thr;
and direct equivalents thereof.

Alternatively, a CD25 binding molecule for use to ameliorate disease symptoms of rheumatoid arthritis may be selected from a single chain binding molecule which comprises an antigen binding site comprising
a) a first domain comprising in sequence the hypervariable regions CDR1, CDR2 and CDR3, said hypervariable regions having the amino acid sequences as shown in Seq. Id. No. 1 in EP 449769,
b) a second domain comprising in sequence the hypervariable regions CDR1', CDR2' and CDR3', said hypervariable regions having the amino acid sequences as shown in Seq. Id. No. 2 in EP 449769 and
c) a peptide linker which is bound either to the N-terminal extremity of the first domain and to the C-terminal extremity of the second domain or to the C-terminal extremity of the first domain and to the N-terminal extremity of second domain;
and direct equivalents thereof.

As it is well known, minor changes in an amino acid sequence such as deletion, addition or substitution of one or several amino acids may lead to an allelic form of the original protein which has identical or substantially identical properties. Thus, by the term "direct equivalents thereof" is meant either any single domain CD25 binding molecule (molecule X)
(i) in which the hypervariable regions CDR1, CDR2 and CDR3 taken as a whole are at least 80 % homologous, preferably at least 90 % homologous, more preferably at least 95 % homologous to the hypervariable regions as shown in Seq. Id. No. 1 in EP 449769 and,
(ii) which is capable of inhibiting the binding of IL-2 to its receptor substantially to the same extent as a reference molecule having framework regions identical to those of molecule X but having hypervariable regions CDR1, CDR2 and CDR3 identical to those shown in Seq. Id. No. 1 in EP 449769;
or any CD25 binding molecule having at least two domains per binding site (molecule X')
(i) in which the hypervariable regions CDR1, CDR2, CDR3, CDR1', CDR2' and CDR3' taken as a whole are at least 80 % homologous, preferably at least 90 % homologous, more pre-

ferably at least 95 % homologous to the hypervariable regions as shown in Seq. Id. No. 1 and 2 in EP 449769 and

(ii) which is capable of inhibiting the binding of IL-2 to its receptor substantially to the same extent as a reference molecule having framework regions and constant parts identical to molecule X' but having hypervariable regions CDR1, CDR2, CDR3, CDR1', CDR2' and CDR3' identical to those shown in Seq. Id. No. 1 and 2 in EP 449769.

This last criterion may be conveniently tested in various assays as described in EP 449769.

Most preferably, the chimeric CD25 antibody for use to ameliorate disease symptoms of rheumatoid arthritis comprises at least

a) one heavy chain which comprises a variable domain having an amino acid sequence identical or substantially identical to that shown in Seq. Id. No. 1 in EP 449769 starting with amino acid at position 1 and ending with amino acid at position 117 and the constant part of a human heavy chain; and

b) one light chain which comprises a variable domain having an amino acid sequence identical or substantially identical to that shown in Seq. Id. No. 2 in EP 449769 starting with glutamic acid at position 1 and ending with glutamic acid at position 104 and the constant part of a human light chain.

The constant part of a human heavy chain may be of the $\gamma 1$, $\gamma 2$, $\gamma 3$, $\gamma 4$, μ, $\alpha 1$, $\alpha 2$, δ or ε type, preferably of the γ type, more preferably of the $\gamma 1$ type, whereas the constant part of a human light chain may be of the κ or λ type (which includes the $\lambda 1$, $\lambda 2$ and $\lambda 3$ subtypes) but is preferably of the κ type. The amino acid sequence of all these constant parts are given in Kabat et al., Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, Public Health Service, NIH..

The most preferred CD25 binding molecule is basiliximab.

A CD25 binding molecule for use to ameliorate disease symptoms of rheumatoid arthritis may be produced by techniques disclosed for example in EP 449769, in particular in Examples 1 to 5 of EP 449769.

Therefore the invention also provides

(i) a method of amelioration of disease symptoms of rheumatoid arthritis which comprises administering a therapeutically effective amount of a CD25 binding molecule as described above to a patient in need of such amelioration.

(ii) a pharmaceutical composition for amelioration of disease symptoms of rheumatoid arthritis which comprises a CD25 binding molecule as described above and a pharmaceutically acceptable carrier or diluent.

(iii) a CD25 binding molecule as described above for use in the manufacturing of a medicament for use in the method as described in (i).

(iv) a method as described in (i) comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of a CD25 binding molecule as described above and a further drug substance, said further drug substance being selected from a group comprising immunosuppressives such as cyclosporin A; methotrexate; azathioprine; glucocorticoids comprising prednisone; slow acting anti-rheumatic drugs comprising chloroquine; and d-penicillamine.

For the use in accordance with the invention, the appropriate dosage will, of course, vary depending upon, for example, the particular molecule to be employed, the host, the mode of administration and the severity of the condition being treated and the effects obtained. Satisfactory results are generally indicated to be obtained at dosages from about 0.1 mg to about 100 mg. Administration may be in a single dose or in several doses over a period of time as long as may be indicated in relation to the time a rheumatoid event may last, for example a dose of 10 to 100 mg may be administered every four weeks up to a time an amelioration of the symptoms may be visible. The CD25 binding molecule is conveniently administered parenterally, normally intravenously, for example, into the antecubital or other peripheral vein. An exemplary dosing regimen is intravenous administration of 60 mg at the beginning, 40 mg 28 days later, followed by another 40 mg 56 days later.

Pharmaceutical compositions of the invention may be manufactured in a conventional manner as described, e.g. in EP 449769.

If the CD25 binding molecule is co-administered with a further drug substance both may be packaged separately within the same container, with instructions for mixing or concomitant administration. Examples of kits include for example a multi-barrelled syringe or a twin pack containing separate unit dose forms.

Murine monoclonal antibodies suitable for use together with the CD25 binding molecule to ameliorate disease symptoms of rheumatoid arthritis are described in EP 449,769.

The intravenous infusions may be prepared as follows: the lyophylized antibodies are mixed together and dispersed into 100 ml sterile buffered saline containing 4.5% wt. of human albumin. This saline dispersion may be administered to the patients over a 30 minute period. The patients also receive standard cyclosporin therapy.

Investigations so far indicate that the administration of the CD25 binding molecules is free from unacceptable side-effects at the dosage levels employed. Particularly the preferred one, basiliximab, is safe, approved by the Federal Drug Administration (FDA) of the United States and is commercially available.

The usefulness of the CD25 binding molecule to ameliorate disease symptoms of rheumatoid arthritis is shown in the following clinical example.

60 patients with active rheumatoid arthritis are enrolled who have a partial response to methotrexate alone. A partial response to methotrexate alone is defined as the presence of at least 6 swollen and 9 tender joints and C-reactive protein level >20 mg/l in patients who have been receiving a stable, maximally tolerated methotrexate dose (not exceeding 20 mg/week) for at least 3 months prior to the screening visit (Week -2).

These 60 patients are randomized to one of two treatment groups; those receiving methotrexate plus basiliximab and those receiving methotrexate plus placebo. The methotrexate dose and route of administration remains stable during the course of the study. The patients are also on stable doses of nonsteroidal anti-inflammatory drugs and prednisone. Basiliximab is administered intravenously at a dose of 60 mg on Day 0, 40 mg on Day 28 and 40 mg on Day 56. Patients are evaluated at Weeks 2, 4, 6, 8, 10 and 12 for safety, efficacy and disease outcome.
The primary efficacy outcome measure is the attainment of ACR (American College of Rheumatology) criteria for improvement of rheumatoid arthritis at Week 12. ACR (20) criteria defines improvement as 20% improvement in the number of tender and swollen joints, in addition to 20% improvement in at least three of five variables (degree of disability, HAQ

(Health Activity Questionnaire); patient global assessment; physician global assessment; pain and C-reactive protein).

Patients receiving basiliximab show an amelioration of the symptoms as compared to patients receiving placebo.

Claims

1. The use of a CD25 binding molecule which comprises at least one antigen binding site comprising at least one domain which comprises in sequence, the hypervariable regions CDR1, CDR2 and CDR3; said CDR1 having the amino acid sequence Arg-Tyr-Trp-Met-His, said CDR2 having the amino acid sequence Ala-Ile-Tyr-Pro-Gly-Asn-Ser-Asp-Thr-Ser-Tyr-Asn-Gln-Lys-Phe-Glu-Gly, and said CDR3 having the amino acid sequence Asp-Tyr-Gly-Tyr-Tyr-Phe-Asp-Phe; or direct equivalents thereof to ameliorate disease symptoms of rheumatoid arthritis.

2. A method for amelioration of disease symptoms of rheumatoid arthritis, which method comprises administering to said subject a therapeutically effective amount of a CD25 binding molecule e.g. substantially as hereinbefore defined and described.

3. A CD25 binding molecule e.g. substantially as hereinbefore defined and described for use in the manufacturing of a medicament for use in the method according to claim 2.

4. A composition comprising a CD25 binding molecule e.g. substantially as hereinbefore defined and described for use in the method according to claim 2 or in the manufacturing according to claim 3.

5. A method according to claim 2 comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of a CD25 binding molecule, e.g. substantially as hereinbefore defined and described and a further drug substance, said further drug substance being selected from a group comprising immunosuppressives such as cyclosporin A; methotrexate; azathioprine; glucocorticoids comprising prednisone; slow acting anti-rheumatic drugs comprising chloroquine; and d-penicillamine.